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BIOREMEDIAL APPROCH TO DEGRADE PHYSICO-CHEMICAL CHARACTERISTICS BY INDIGENOUS MICROBES IN PAPER AND PULP INDUSTRY

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ABSTRACT

Recent studies have found paper and pulp industries responsible for polluting the environment in India by releasing hazardous liquids which contain heavy metals and other toxicants. The effluents released from these industries, pollute the water bodies. The polluted water bodies contain compounds which are toxic to aquatic flora and fauna as well as have a strong mutagenic effect. Bioremediation may serve as an appropriate method to reduce the physico-chemical parameters to a prescribed Limit by CPCB. Biological treatment has been reported efficacious in reducing the organic load and toxic effects of kraft mill effluents. The present investigation was aimed to degrade physic-chemical characteristics from effluent generated by Pulp and Paper industry. The physico-chemical analysis of effluents showed that these characteristics were notably high which was not permissible by CPCB and ISI. Based on the isolation, identification and biochemical characterization studies the isolated bacterial strain was identified as Bacillus sp.Simulated approach was utilized to monitor Physicochemical properties (DO,COD, BOD, Alkalinity, Acidity, Chloride, Hardness, Nitrate, Phosphate) after bacterial treatment. A reduction in all the physico-chemical properties was observed with post bacterial treatment which was in accordance with the standards prescribed.

KEYWORDS: Paper and Pulp Industry, Bioremediation, Bacteria, Physico-chemical Characteristics

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Original Article

INTRODUCTION

In the present scenario, the industrial development has been grown up to a height. The successful attempt of green revolution and industrialization has contributed towards the progress of mankind along with certain consequences which had led to environmental pollution to a great extent. One such main stream industry recognized by Ministry of Environment and Forest, Government of India is pulp and paper industry which has led to a very prominent increase in the rate of water pollution. Pulp and paper industry is one of the major water consuming industries (Trivedi and Raj, 1992), about 80% of this water reappear as wastewater and is usually discharged back into the streams leaving it untreated. Lakes and other water bodies are currently being affected by both natural and anthropogenic activities, which degrade their quality, and push them to the brink of extinction in the process of planned development, giving rise to the need for suitable conservation strategies. Unfortunately, over the years, mere attention has been paid to wetland losses worldwide, including India. The deterioration of the lakes and water bodies has altered their functions, affecting the ecological balance. Generally, lake functions directly relate to their physico-chemical and biological integrity. The altered physicochemical properties have caused a significant loss to the quality of water as well as to the aquatic life residing there. The altered physicochemical properties such as pH, BOD, COD, DO&Salinity has caused a significant balance to the

ecosystem inthe particular water source. The correct balance of physicochemical parameters is required for the proper maintenance of water resources and proper functioning of the eco system. Physicochemical parameters play an important role in maintaining a proper balance between the organic and inorganic properties of a particular water resource. Physicochemical parameters along with the biological parameters create an appropriate balance or a particular water resource by maintaining an appropriate temperature and many physical properties. (Gale *et al*. 2003).

Bioremediation means the resolution of problem through biological means and to use live organisms to solve a problem which affects the environment such as contaminated soil or groundwater. It can be defined as a process which tends to use metabolic pathways of microbes fungi, green plants or enzymes on the contaminated or altered site to return them back to its less toxic or original form naturally. It may be employed to attack specific types of contamination or contaminants such as reduction of chlorinated hydrocarbons by bacteria. Bioremediation is an approach which involves the use of organisms to remove or reduce pollutantsin their least toxic form. The waste or toxic material can be collected from the polluted sites & the bioremediation with the bioremediation with the requisite microorganism can be carried out at designed places. The term Bioremediation is being introduced to describe the process of using biological agents such as micro-organisms to reduce toxic waste from the environment. Bioremediation could be a very prominent and most effective management tool to manage the quality ofthe environment and to recover a resource from its contaminated form. It has been reported that bioremedial approach has been used many times at a number of sites worldwide, including Europe, with varying results on board. (Kumar A 2010) Bioremediation can be classified into *in situ* or *ex situ*. *In situ* bioremediation involves treatment ofthe contaminated material on-site, whereas *ex situ* involves the removal of the contaminated resource and its treatment in a defined facility. Researchershave reportedthat there are very few environments where microbes were unable to survive, adapt, and indeed, thrive.

The microbes are able to modify near infinite combinations of electron donors and acceptors which drive their metabolism. In addition to these redox reactions, they have also equipped themselves with arsenal of other strategies to detoxify a particular environment. There are many factors which govern the control of complex process of bio-remediation such as the existence of a microbial population which could degrade the pollutants, avail of contaminants to the microbial population, and other environmental factors such as type of soil, temperature, pH, presence of oxygen and nutrients.

MATERIAL AND METHODOLOGY

Collection of the Affected Water Samples

Collection of the affected water samples was done from Century Paper and PulpIndustry Sanganer Area, Jaipur. Along with it the water sample which was not being affected by any of the bacterial species was taken as a negative control for the study.

Physico-Chemical Analysis of the Water Samples Prior and After Bio-remedial Setup

The samples of effluents were analyzed for physico-chemical parameters before and after the bioremedial setup. The parameters temperature**,** DO was observed during the collection of the samples prior to the other physico-chemical analysis. The other parameters like pH, total alkalinity, Acidity, COD, BOD, were analyzed as per the standard procedures.

Isolation of Potential Bacterial Strain

The water sample collected was subjected to bacteriological analysis using culture dependent approach. The sample was screened with Chromium metal. The bacterial isolates which showed growth in the presence of Chromium

were selected for their biodegradation potential as they had the ability to degrade Chromium. A total of 2 isolates were screened and were designated as C1 and C2. They were identified according to culture dependent approach. A series of biochemical tests were used to identify them (V.P kesalkar et al 2012.)

Identification of the Isolated Bacteria

For the identification and isolation of the bacterial strain microscopic and biochemical characterisation techniques were being used. Simple and gram staining were used for microbial identification. Later for the confirmatory study biochemical characterisation tests such as gelatine hydrolysis, catalase test, starch hydrolysis, citrate test & MR-VP Test were carried out.(Capucinno And shermann 2001).

Setting up of the Bioremedial Setup

A bio-remediation setup was apparatus was being setup and the affected water samples were inoculated with the isolated bacteria and it was incubated for 15 days. After 15 days the samples were being checked for the reduction in the physico-chemical parameters by standard procedures.

RESULT AND DISCUSSIONS

Bioremediation served as a very effective way in the reduction of physico-chemical parameters for the affected water sample and reduce it back considerably hence improving the quality of water by certain extent. The study was carried out to assess the quality of untreated effluent water released from paper and pulp industry. The data obtained for the physico-chemical properties of the water sample, when compared, were not in the range of permissible limits (Richa et.al.2012) indicating that the water was polluted. The environmental conditions also play a vital role in the process of bioremediation as micro organisms need optimum temperature, pH, Nutrition for their optimum growth and functionality. From Physicochemical characterisation of the water sample it is clear that there is a difference in the quality of the water by comparing the before and after results of the bioremediation.(Table: 1 parameters Before Bioremediation) (Table: 2 Parameters after Bioremediation) There was a prominent change in the parameters such as pH, COD,BOD,DO, Sulphate, Nitrate, oil&grease, acidity, alkalinity,chloride, hardness, phosphate, TDS.

Table 1: A Table Representing Physicochemical Parameters before Bioremediation

Parameters	Units	Physicochemical Parameters Before Bioremediation	CPCB Standards
1. Colour	-	Colourless	-
2. pH	-	4.8	5.8-9.0 [A]
3. Temperature	C°	28.5	45 [A]
4. Total Dissolved Solids (TDS)	Mg/l	616	200 [A]
5. Suspended Solids (SS)	Mg/l	150	200 [A]
6 Electrical Conductivity (EC)	Mg/l	1231	-
7. Dissolved Oxygen (DO)	Mg/l	1.76	4 [A]
8. Biological Oxygen Demand (BOD)	Mg/l	450	30 [A]
9. Chemical Oxygen Demand (COD)	Mg/l	1400	350 [A]
10. Hardness	Mg/l	1461	-
11. Salinity	Mg/l	595.6	500 [A]
12. Acidity	Mg/l	238	-

Table 1: Contd.,			
13. Alkalanity	Mg/l	104	200 [B]
14. Phosphate	Mg/l	2	5 [B]
15. Sulphate	Mg/l	316.47	565 [B]
16. Nitrate	Mg/l	30	45 [B]
17. Oil & Grease	Mg/l	0.131	10 [B]

Table 2: A Table Representing Comparison of Physicochemical Parameters before Bioremediation and after Bioremediation of C_1 Strain

Parameters	Units	Physicochemical Parameters before Bioremediation	Physicochemical Parameters after Bioremediation (C_1, C_2, C_3 Strain)
1. Colour	-	Colourless	Colourless
2. pH	-	4.8	6.5
3. Temperature	Cº	28.5	28.5
4. Total Dissolved Solids (TDS)	Mg/l	616	512
5. Suspended Solids (SS)	Mg/l	150	70
6 Electrical Conductivity (EC)	Mg/l	1231	1610.49
7. Dissolved Oxygen (DO)	Mg/l	1.76	2.9
8. Biological Oxygen Demand (BOD)	Mg/l	450	90
9. Chemical Oxygen Demand (COD)	Mg/l	1400	290
10. Hardness	Mg/l	1461	998
11. Salinity	Mg/l	595.6	356
12. Acidity	Mg/l	238	190
13. Alkalanity	Mg/l	104	80
14. Phosphate	Mg/l	2	1.9
15. Sulphate	Mg/l	316.47	217
16. Nitrate	Mg/l	30	21
17. Oil & Grease	Mg/l	0.131	0.115

Later The isolation (Figure 1) morphological identification(Gram Staining) (Figure 2) BiochemicaI identification tests (Figure 3) strains show that the degrading microorganisms belonged to *Bacillus sp*

Figure 1: Pure Colonies of Isolated Bacterial Species on Nutrient Agar

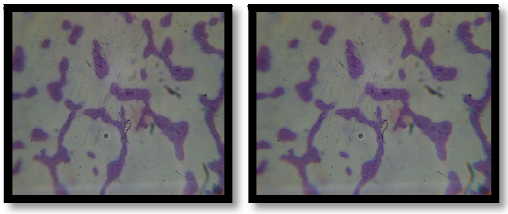


Figure 2: Gram Staining of Isolated Bacterial Strains *(Bacillus sp)*

Figure 3: Biochemical Tests for the Isolates Screened from the Affected Water Sample

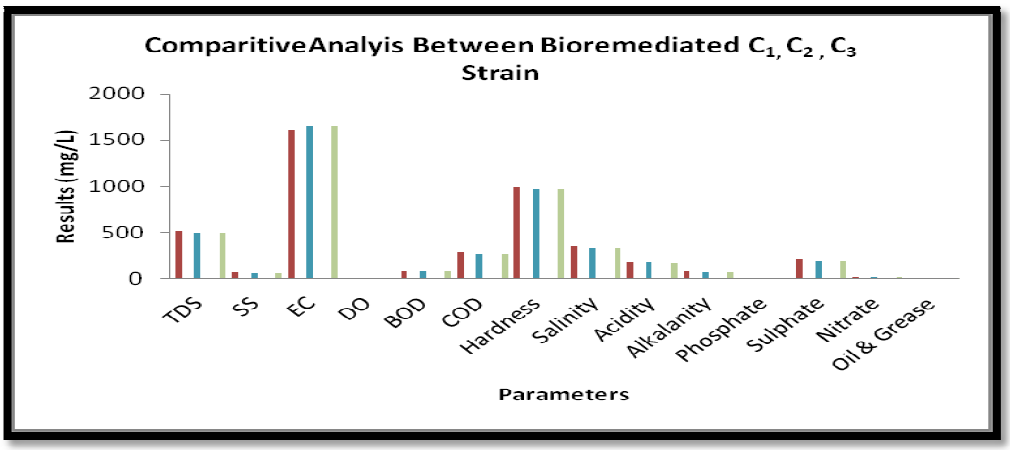
The degradation was seen in the c1 strain and c2 strain the degradation was more in c2 strain as the bio remedial setup varied with the concentration of the bacterial innoculum. The degradation in the physic chemical parameters took place by enzymatic reaction which led to decrease in various physicochemical parameters.

Table 3: Table Representing Morphological Characters of the Culture

S. No	Isolate	Form	Edge	Elevation	Surface	Colour
1.	C1	Circular	Entire	Raised	Smooth	Creamish
2.	C2	Irregular	Undulate	Flat	Rough	Creamish
3.	C3	Circular	Entire	Raised	Smooth	White

Table 4: Table Representing Morphological Characters of the Culture

S. No	Test	C1	C2	C3
1.	Starch Hydrolysis	Negative	Positive	Negative
2.	Catalase	Positive	-	Positive
3.	Citrate Utilization	-	Positive	-
4.	MR-VP	-	Negative	-
Conclusion	-	*Bacillus sp*	*Bacillus sp*	*Bacillus sp*



Graph 1: A Graph Representing Comparative Analysis of Various Physicochemical Parameters for C_1 C_2 & C_3 Strain

DISCUSSIONS

The present study was carried out to assess the quality of effluent water from paper and pulp industry. The data obtained from the physico-chemical properties of the water sample, when compared, were not in the range of permissible limits (Richa et. al. 2012) indicating that the water was polluted. Nutrient agar was used in an enrichment technique supplemented with adequate amount of nutrients. The data forecasts that isolated microorganisms belonged to, *Bacillus sp.* and were studied for their biodegradation potential for physicochemical parameters. Most literature found that effective indigenous isolates release organic acids and enzymes to utilize and degrade xenobiotic compounds.(Monica et.al.2011).Virendra Kumar (2011) also found similar results with *Klebsiella sp.* (99%, accession no NR_074913.1), *Alcaligens sp*. (99%,accession No. NR_025357.1) and *Cronobacter sp*. (97%, accession No.NR_102490.1). The results of survivability showed that the bacteria were not only effective but also dominant irrespective of the other strains present in the effluent. Intense research in this area confirms that besides bacteria, other microorganisms, including fungi and algae, can be used. According to Indhumathi P. (2014). Chlorella vulgaris is a cheap and effective adsorbent for the reduction of physicochemical parameters of giving waste water samplewithout requiring any prior treatment. In the Present study biodegradation process was conducted Titirimetrically (K Murugesan, 2003). In this study we found that reduction of physicochemical parameters with 0.1% of inoculum, 95% reduction of oil was found in *Bacillus sp.*with. Thus, the results obtained from this research are promising in future project to use this bacteria as a biological agent to degrade hexavalent Chromium from industrial effluent of paper and pulp industry. These isolates can be utilized for degradation of chromium in textile industry, steelindustry and paint industry with the eco-friendly approach.

CONCLUSIONS

Within the underlying soil the contamination persists to a degree which poses a major effect on the natural resources and hence contamination is correlated with the degree of industrialization and intensity of chemical usage. One of the major concerns is over water contamination which primarily affects health aspects via direct contact with the contaminated soil as well as the contamination of the water supplies which further contaminates the underlying soil. Textile dyes and petroleum components which contain harmful hydrocarbons, carcinogens and toxic heavy metals are very harmful to the human health and the environment as well. Bacterial communities tend to utilize the contaminants present in the soil as a potent source of carbon and energy which leads to the ambient production of a wide range of contaminants such as petroleum and poly-aromatic hydrocarbons. The production of these contaminants are one of the most affecting and limiting factors for an efficient bioremediation. Other factors such as nutrient availability, moisture content, pH of the soil, C/N ratio also contributes towards minimal degradation of xenobiotic compounds.Oil adapted bacterial genera were isolated and identified from the test water sample: they were *Bacillus sp.* and *Staphylococcus sp...* It is therefore recommended that ability of the isolated and identified bacteria to bio remediate or utilize hydrocarbons, especially used petroleum oil should be investigated.

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